1 ISOLATING DNA USING THE DNA IQ™ ISOLATION SYSTEM

When the DNA examiner has completed his/her analysis, all remaining evidence samples, including extracted DNA which has been dried down using a DNA concentrator/evaporator (Refer to Appendix K, Procedure For Drying Down And Resolubilizing Extracted DNA, of Section III of the Forensic Biology Section Manual) or other suitable methods, will be returned to the submitting agency. However, amplified DNA samples WILL NOT be returned to the submitting agency, but instead will be discarded in the designated area to prevent possible transfer of amplified DNA to the remaining evidence samples. If the biological sample deposited on the evidence is consumed during the analysis then the extracted DNA sample and the cutting (in separate tubes) will be returned to the submitting agency with the evidence. Proper case file documentation and chain of custody documentation for these samples must be maintained.

DNA for PCR amplification and analysis may be extracted from bloodstains, sperm cells, buccal cells, hair, tissue, bone, and other samples. Slightly different extraction procedures are required for each type of specimen and therefore are outlined in this chapter.

It is important to handle all samples aseptically to prevent contamination by extraneous DNA. It is also important to prepare evidence samples at a separate time and/or space from reference samples to prevent possible cross-contamination.

NOTE: For tracking purposes the samples will be listed on the worksheet in the order in which they were processed/handled and will be processed in accordance with the procedures outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section VI - Quality Assurance Program DNA Typing of Biological Materials, Chapter 6, Dedicated PCR Facilities.

Special Precautions:

- The DNA extraction and PCR setup of evidence samples will be performed at a separate time or space from the DNA extraction and PCR setup of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.
- DNA extraction from samples containing high levels of DNA (for example, tissue) will be performed separately from samples expected to contain low levels of DNA (single hairs, small bloodstains, etc.) to minimize the potential for sample-to-sample contamination.
- Disposable gloves will be used at all times. Gloves will be changed frequently to avoid sample-to-sample contamination with DNA and whenever moving between work areas. Gloves will be changed if suspected direct contamination has occurred from the sample DNA.
- Scissors will be thoroughly cleaned with a 10% solution of bleach or a solution that will remove/degrade the DNA after cutting each item/stain. Subsequently use Isopropyl Alcohol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces. A fresh scalpel blade may also be used to cut each item/stain.
- A clean cutting surface will be used for each piece of evidence.
- Disposable plugged pipette tips and microcentrifuge tubes will be used.

- Pipette tips will be changed between samples.

- Reagents will be stored in small quantities to reduce the risk of possible contamination to the stock solution.

- To avoid splashing and minimize aerosols, all liquid will be centrifuged to the bottom of the closed tube before the tube is opened.

- Reagent blank controls will be included with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.

- All work surfaces will be thoroughly cleaned with a 10% bleach solution or a solution that will remove/degrade the DNA. Subsequently use Isopropyl Alcohol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces before setting up the DNA Extraction Work Area. Disposable bench paper will be used to prevent the accumulation of human DNA on permanent work surfaces.

- The quantity of samples handled during a single analysis will be limited to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.

- A dedicated lab coat will be worn for pre-amplification sample handling when working in the DNA Extraction Work Area.

- A dedicated lab coat will be worn when working with amplified DNA in the PCR Post Amplification Work Area.

1.1 TECHNICAL NOTES

1.1.1 The DNA IQ™ Isolation System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less then 50 ng of DNA.

1.1.2 The DNA IQ™ Isolation System will isolate genomic DNA in general and is not human specific.

1.1.3 A random sample will be run with each set of convicted offender and arrestee sample extractions to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly. If a sample must be re-extracted a new random sample must be extracted along with the sample.

1.1.4 Routinely the DNA IQ™ extraction method is used to isolate DNA from blood/buccal samples obtained in criminal cases and the QIAGEN® BioRobot™ 9604 is used to isolate DNA from convicted offenders blood/buccal or arrestee buccal samples in accordance with the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section V - QIAGEN® BioRobot™ 9604 Procedure Manual. However, when necessary convicted offender blood/buccal and arrestee buccal samples may also be extracted using the procedures outlined in this manual.

1.2 EQUIPMENT

1.2.1 Heat block or incubator, 37ºC

1.2.2 Heat block or incubator, 56ºC

1.2.3 Microcentrifuge

1.2.4 Vortex mixer

1.2.5 Scissors

1.2.6 Tweezers

1.2.7 Microcentrifuge tube rack

1.2.8 Pipettes - 10 µL, 100 µL, 200 µL, and 1000 µL

1.2.9 Refrigerator/Freezer

1.2.10 Scalpel and blades (for DNA extraction from swabs)

1.2.11 Stereo microscope (for DNA extraction from hair)

1.2.12 Beaker, 50 mL (for DNA extraction from hair)

1.3 MATERIALS

1.3.1 Microcentrifuge tubes, 1.5 mL

1.3.2 Transfer pipettes

1.3.3 Sterile ART tips for pipettes - 10 µL, 100 µL, 200 µL, and 1000 µL

1.3.4 Microcentrifuge tube lids

1.3.5 Kimwipes

1.3.6 Spin-Ease basket (Optional)

1.3.7 Gloves

1.4 REAGENTS

1.4.1 Proteinase K - 20 mg/mL (Keep on ice.)

1.4.2 TNE

1.4.3 20% Sarkosyl

1.4.4 0.39*M* Dithiothreitol (DTT)

1.4.5 Sterile Type I Water

1.4.6 PCR Digestion Buffer

1.4.7 95% Ethanol

1.4.8 DNA IQ™ System Reaction Kit

1.4.9 DNA IQ™ Lysis Buffer

1.4.10 DNA IQ™ Wash Buffer

1.4.11 DNA IQ™ Elution Buffer

1.4.12 DNA IQ™ Resin

1.5 DNA IQ™ EXTRACTION METHOD FOR BUCCAL CELL TYPE SAMPLES AND BLOODSTAINS

The portion size of the swab or bloodstain removed for DNA extraction should be judged based on a number of criteria, such as whether the stain appears dilute (for bloodstains) or if the sample may be heavily soiled or possibly degraded. Examine the bloodstain or buccal swab and remove a reasonable portion for DNA extraction. For example, only a 3 mm^2 section may be all that is necessary to remove from a heavily bloodstained item.

NOTE: Process a reagent blank along with each set of samples.

1.5.1 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If a stain is smeared over a large area of fabric, more than one microcentrifuge tube may be needed to extract the stain. If the sample is a buccal swab, remove a small portion of the swab and place into a labeled microcentrifuge tube.

NOTE: Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.5.2 Add 250 uL of DNA IQ™ Lysis buffer. If the biological sample is dispersed over a large area of the substrate, add up to 325 uL of DNA IQ™ Lysis buffer to the microcentrifuge tube.

1.5.3 Vortex vigorously for 20-30 seconds, then pulse spin to force the cutting into liquid.

1.5.4 Place the microcentrifuge tube into a 56ºC heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper, place the microcentrifuge tube into a 95° C heat block for a minimum of 30 minutes.

1.5.5 Vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube. If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence.

NOTE: Samples can be left at room temperature in DNA IQ™ Lysis buffer for up to 24 hours after heating and centrifugation before proceeding to the BioMek® 2000 Automation Workstation. If the samples are stored in a refrigerator before proceeding to Section 2, Preparation of the BioMek® 2000 Automation Workstation, place the samples in a 56ºC heat block for 5 minutes to resolubilize the samples.

1.5.6 Proceed to Section 2, Preparation of the BioMek® 2000 Automation Workstation.

1.6 DNA IQ™ EXTRACTION METHOD FOR MIXED BODY FLUID STAINS (DIFFERENTIAL PROCEDURE)

1.6.1 Cut an approximate 3 mm^2 stain or a portion of a cotton swab proportional to the number of sperm identified. Place the stain into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If the stain is smeared over a large area on the fabric or the body fluids on a swab are weak and more than one swab must be used, more than one microcentrifuge tube may be needed to extract the stain.

1.6.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube during the purification step.

NOTE: Process a reagent blank along with each set of samples.

1.6.2 Add:

400 µL TNE
25 µL 20% Sarkosyl
75 µL Sterile Type I Water
5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.6.3 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.6.4 Place the tube into a 37ºC incubator or heat block for a minimum of 2 hours.

1.6.5 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube.

1.6.6 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NON-SPERM FRACTION.

1.6.7 At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready, and then proceed to Section 2, Preparation of the BioMek® 2000 Automation Workstation, with both the sperm and non-sperm fractions.

1.6.8 Remove and discard the old lid and cutting from the original tube containing the pellet. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new colored lid on the tube. This tube contains the SPERM FRACTION.

1.6.9 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000

rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.6.10 Repeat the wash in step 1.5.16 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. After the final wash, remove all but 50 µL of the wash buffer and discard. The sperm cells are not lysed at this point. The sperm cells are lysed during the isolation step using the BioMek® 2000 Automation Workstation

NOTE: If necessary the sample(s) once capped can remain at room temperature overnight or be stored in a refrigerator before proceeding to Section 2. If stored in a refrigerator place the samples in a 56ºC heat block for 5 minutes to resolubilize the DNA before proceeding.

1.6.11 Proceed to Section 2, Preparation of the BioMek® 2000 Automation Workstation, with both the sperm and non-sperm fractions.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

1.7 DNA IQ™ EXTRACTION METHOD FOR HAIR AND LOW LEVEL SAMPLES

NOTE: This procedure may also be used for samples that are believed to have a low concentration of DNA (i.e., envelopes, stamps, cigarette butts) or highly concentrated bloodstain (i.e., dried blood flakes).

1.7.1 Follow the steps outlined below for the extraction of hairs. Proceed to step 1.6.2 for all other sample types:

1.7.1.1 Using clean tweezers, place the hair on a clean piece of white or black paper, whichever is appropriate, and examine under a stereo microscope for the presence of sheath material. Note the presence of any body fluids on the hair.

1.7.1.2 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I Water in a clean 50 mL beaker. If the hair contains a biological fluid that is important to the investigation DO NOT wash the hair.

1.7.1.3 Return the hair to the stereo microscope. Use a clean scalpel blade to cut a 0.5 to 1 cm portion from the root end of the hair and then place the hair root into a 1.5 mL microcentrifuge tube and proceed to step 1.6.3.

NOTE: Process a reagent blank along with each set of samples.

1.7.2 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid

1.7.3 Add to the 1.5 mL microcentrifuge tube:

37.5 µL TNE
12.5 µL 20% Sarkosyl
10.0 µL 0.39*M* DTT
32.5 µL Sterile Type I Water
10.0 µL Proteinase K

in proportional amounts to saturate the cutting. NOTE: the maximum volume of sample loaded into the deep well plate is 100 µL.

1.7.4 Mix by hand or lightly vortex, then pulse spin the microcentrifuge tube to force the sample into the liquid.

1.7.5 Place the tube into a 56ºC incubator or heat block for minimum of 1 hour.

1.7.6 Pulse spin the tube in a microcentrifuge for 10 seconds to force the condensate to the bottom of the tube.

1.7.7 Proceed to Section 2, Preparation of the BioMek® 2000 Automation Workstation.

♦**END**